



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification: C07K 14/00</p>	<p>A2</p>	<p>(11) International Publication Number: WO 98/02453</p> <p>(43) International Publication Date: 22 January 1998 (22.01.98)</p>																																																																																																																																	
<p>(21) International Application Number: PCT/BE97/00084</p> <p>(22) International Filing Date: 15 July 1997 (15.07.97)</p> <p>(30) Priority Data: 96870092.2 15 July 1996 (15.07.96) EP <i>(34) Countries for which the regional or international application was filed:</i> AT et al. 96870121.9 19 September 1996 (19.09.96) EP <i>(34) Countries for which the regional or international application was filed:</i> AT et al.</p> <p>(71) Applicant (for all designated States except US): UNIVERSITE LIBRE DE BRUXELLES [BE/BE]; Avenue F.D. Roosevelt 50, B-1050 Brussels (BE).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): GOURLET, Philippe [BE/BE]; Avenue Guillaume Stassart 103, B-1070 Brussels (BE). ROBBERECHT, Patrick [BE/BE]; Avenue de Visée 65, B-1170 Brussels (BE). VANDERMEERS, André [BE/BE]; Avenue du Pesage 9, B-1050 Brussels (BE). WOELBROECK, Magali [BE/BE]; Rue Démosthène 63, B-1070 Brussels (BE).</p>	<p>(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B-1080 Brussels (BE).</p> <p>(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>																																																																																																																																		
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<p>(57) Abstract</p> <p>The present invention is related to a peptidic ligand having a higher selectivity for the VIP₁ receptor than for the VIP₂ receptor. The present invention is also related to the diagnostic device, the pharmaceutical composition comprising said ligand and to their use.</p>																																																																																																																																			

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10 PEPTIDIC LIGANDS HAVING A HIGHER SELECTIVITY FOR THE VIP₁
RECEPTOR THAN FOR THE VIP₂ RECEPTOR

Field of the invention

The present invention is related to new peptidic ligands having a selective affinity for the VIP₁ receptor and to the pharmaceutical composition comprising
15 them. The present invention is also related to the use of said compound and said composition as therapeutic agent, specially in the treatment of bronchoconstrictive disorders. The present invention is also related to said
20 ligands of VIP₁ receptor being labelled, and incorporated into a diagnostic device which could be used in imagery or as diagnostic tool.

Background of the invention

25 The study of structure-activity relationships of Vasoactive Intestinal Polypeptide (VIP), Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) and secretin has revealed the existence of domains responsible for high affinity binding, coupling to the
30 effector system and discrimination between receptor subclasses.

- a) The N-terminal sequence is required for a high affinity binding as well as for an efficient coupling to the effector (1-10).
- b) The C-terminal sequence is also necessary for a high affinity binding [11]. However, C-terminally truncated peptides which retain the ability to bind to the receptors have a full biological activity.
- c) The central part (10-21) is usually considered as a spacer domain adopting a α -helical conformation [12,13] and the side chains of the central residues, as not essential for receptor binding (11-13).

Variant VIP synthetic analog peptides including cyclic VIP analog peptides were described in the US Patent US-5234907 and in the European Patent Applications EP-0405242 and EP-0536741.

Vasoactive Intestinal Polypeptide (VIP) effects are mediated by high affinity Gs protein coupled receptors. The molecular cloning revealed the existence of two distinct VIP receptors with seven transmembrane helices named the VIP₁ and the VIP₂ receptors (26-30).

VIP₁ and VIP₂ receptors are pharmacologically distinct : the VIP₂ receptor had a higher affinity than the VIP₁ receptor for helodermin and a lower affinity for secretin and GRF (31).

The ligand RO 25-1553 is a long-acting Vasoactive Intestinal Polypeptide (VIP) agonist developed by O'Donnell et al. from Hoffman-La Roche.

The resulting cyclic peptide with a lactam ring between position 21 to 25 was reported to exhibit a high potency, metabolic stability and a long duration of action. In addition to its potent relaxing effect on guinea

pig tracheal smooth muscle and on isolated human bronchial preparations (1), RO 25-1553 also suppresses various pathophysiological features associated with pulmonary anaphylaxis and asthma including airway reactivity, oedema
5 formation and granulocyte accumulation.

Aims of the invention

The present invention aims to provide new ligands having improved selectivity and affinity for the
10 VIP₁ receptor, a diagnostic device and a pharmaceutical composition comprising said ligands, possibly labelled.

A further aim of the invention is to provide new ligands which may be used as therapeutic agent, especially in the treatment of bronchoconstrictive
15 disorders (such as asthma, COPD), of tumours (neuroendocrine tumours, gastroenteropancreatic tumours, colon tumours) and of myocardial infarctions and strokes, the regeneration of the nerves as in post-traumatic injury, as anti-inflammatory and anti-oxidant agent, for the
20 increasing of cells growth, as immuno-modulation agent in the treatment of auto-immune diseases and for reducing side effects in organs transplantation.

Another aim of the invention is to use said specific ligands in a diagnostic device for the
25 identification of specific cancers such as breast and prostate cancers, lung cancers, ovarian cancers, colon cancers, etc.

Another aim of the invention is to provide a tool which allows the identification of other ligands of
30 VIP₁ receptor, which could be used in the treatment and/or the prevention of the above-identified diseases.

A last aim of the invention is to provide a diagnostic device which comprises said labelled ligands and which may be used in the imagery or as a diagnostic tool in order to improve the diagnostic and/or to measure the evolution of the above identified disease.

Summary of the invention

The present invention is related to a new peptidic ligand which presents a high selectivity for the VIP₁ receptor, preferably the mammalian VIP₁ receptor, more preferably the human VIP₁ receptor.

"High selectivity for the VIP₁ receptor" means that the ligand according to the invention is more selective for the VIP₁ receptor than for the VIP₂ receptor, and that said selectivity is 10-fold more important for the VIP₁ than for the VIP₂ receptor, preferably 100-fold more important for the VIP₁ than for the VIP₂ receptor, more preferably 1000-fold more important for the VIP₁ than for the VIP₂ receptor.

Advantageously, the ligand according to the invention presents in position 16 (R₁₆) a basic amino acid, preferably the Arginine (Arg). Preferably, said ligand presents also in position 4 (R₄) an amino acid chosen from the group consisting of Ala, Gly, D-Ala or D-Phe.

The position of the amino acids of the various peptidic ligands according to the invention is compared to the general formula of the known agonists of the VIP receptor(s) as described in the publication of Christophe J. et al. ("Peptidic Hormones as Prohormones : Processing, Biological Activity, Pharmacology". Ellis

Horwood Limited, Chichester (Jean Martinez Ed.), pp. 211-243 (1989)).

The nomenclature used to define the peptidic ligands according to the invention is that typically used
5 in the art wherein the amino group of N-terminus appears to the left and the carboxyl group at the C-terminus appears to the right.

The one-letter or three-letters amino acid symbol are the ones of the IU-PAC-IUB Biochemical
10 Nomenclature Commission.

The peptidic ligand according to the invention can be an agonist, an antagonist or a reverse agonist of the VIP₁ receptor.

Preferably, said ligand is an agonist wherein
15 R₁ = His, R₂ = Ser, R₃ = Asp, R₄ = Xaa, Ala, D-Ala or Gly, R₅ = XAA, R₆ = Phe, R₇ to R₉ = Xaa, R₁₀ = Tyr, R₁₁ to R₁₄ = Xaa, R₁₅ = Lys, R₁₆ = Arg, R₁₇ to R₂₂ = Xaa, R₂₃ = Leu, R₂₄ to R₂₅ = Xaa, R₂₆ and R₂₇ = Xaa or are deleted. Preferably, said agonist is the PG-97-268, which
20 is a synthetic VIP/GRF analog, and having the formula H-S-D-A-V-F-T-N-S-Y-R-K-V-L-K-R-L-S-A-R-K-L-L-Q-D-I-L-NH₂.

Preferably, said agonist presents also a high affinity to the VIP₁ receptor, which is shown by its IC₅₀ value for the human VIP₁ receptor.

25 Advantageously, the IC₅₀ value of said agonist for the VIP₁ receptor is ≤ 30 nM, preferably ≤ 5 nM. More preferably, the IC₅₀ value of the agonist according to the invention is compared to the IC₅₀ value of the VIP ligand for the VIP₁ receptor.

According to another embodiment of the present invention, the peptidic ligand is an antagonist wherein $R_1 = \text{Ac-His}$, $R_2 = \text{D-Phe}$, $R_3 = \text{Asp}$, $R_4 = \text{Xaa}$, Ala, D-Ala or Gly, $R_5 = \text{Xaa}$, $R_6 = \text{Phe}$, R_7 to $R_9 = \text{Xaa}$,
5 $R_{10} = \text{Tyr}$, R_{11} to $R_{14} = \text{Xaa}$, $R_{15} = \text{Lys}$, $R_{16} = \text{Arg}$, R_{17} to $R_{22} = \text{Xaa}$, $R_{23} = \text{Leu}$, R_{24} to $R_{25} = \text{Xaa}$, R_{26} and $R_{27} = \text{Xaa}$ or are deleted. Preferably, said antagonist is the PG-97-269 having the formula $\text{Ac-H-(D-Phe)-D-A-V-F-T-N-S-Y-R-K-V-L-K-R-L-S-A-R-K-L-L-Q-D-I-L-NH}_2$.

10 The above-identified agonist or antagonist comprise 27 amino acids or less.

Advantageously, said antagonist presents also a high affinity for the VIP_1 receptor, measured by its IC_{50} value for the VIP_1 receptor, which is measured by its IC_{50}
15 value for the VIP_1 receptor which is $\leq 100 \text{ nM}$, preferably $\leq 50 \text{ nM}$.

Preferably, the VIP_1 receptor above-described is a mammalian receptor, preferably a human receptor.

The Inventors have tested in vitro the
20 capacity of ligands to occupy the different VIP/PACAP receptor subclasses and to stimulate or inhibit adenylate cyclase activity. The cellular models tested expressed one single receptor subtype : Chinese hamster ovary (CHO) cells transfected with the rat recombinant PACAP I-, rat VIP_1 -,
25 rat VIP_2 -, rat secretin receptor and human VIP_2 receptors; LoVo cells expressing the human VIP_1 receptor. The agonist PG-97-268 had IC_{50} values of binding of 1, 10000, 10000 and 30000 nM for the rat VIP_1 -, VIP_2 -, secretin and PACAP receptors, respectively. It showed an IC_{50} of 0.8 nM for

5 The antagonist PG-97-269 had IC_{50} values of binding of 10, 2000, 2 and 3000 nM on the rat VIP_1 -, VIP_2 -, human VIP_1 -, VIP_2 - receptors, respectively. PG-97-269 had a negligible affinity for the PACAP I receptor subtype. It did not stimulate adenylate cyclase activity, but inhibited competitively effect of VIP on the VIP_1 receptor mediated stimulation of adenylate cyclase activity with K_i values respectively of 15 and 2 nM for the rat and human VIP_1 receptors. Thus PG-97-268 is a highly selective agonist ligand for the VIP_1 receptor and PG-97-269 a highly selective antagonist ligand.

20 The above representative ligands may be readily synthesised by any known conventional procedure for the formation of a peptide linkage between amino acids, including for example any solution phase procedure permitting a condensation between the free alpha amino

25 group of an amino acid residue thereof having its carboxyl group or other reactive groups protected and the free primary carboxyl group of another amino acid or residue thereof having its amino group or other reactive groups protected.

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whereby each amino acid in the desired sequence is added one at a time in succession to another amino acid or residue thereof or by a procedure whereby peptide fragments with a desired amino acid sequence are first synthesised
5 conventionally and then condensed to provide the desired peptide. Said conventional procedure for synthesising the novel ligand of the present invention include for example any solid phase peptide synthesis method. The synthesis of the ligands can be carried out by sequentially
10 incorporating the desired amino acid residues one at a time into the growing peptide chain according to the general principles of solid phase methods [Merrifield, R.B., J. Amer. Chem. Soc. 85, 2149-2154 (1963); Brany et al, The Peptides, Analysis, Synthesis and Biology, Vol. 2, Gross,
15 E. and Meienhofer, J. Ads. Academic Press 1-284 (1980)].

Said ligands may also be protected by various reactive product as described in the European Patent Application EP-0405242 incorporated herein by reference.

The new ligands of the present invention have
20 also improved pharmacological properties and can be used as therapeutic agents.

The new ligands of the invention are preferably used for the treatment of bronchostrictive disorders such as asthma, COPD (Chronic Obstructive
25 Pulmonary Disease). Said ligands may also be used for the treatment of various tumours, preferably chosen among the group consisting of (neuro)endocrine tumours, gastroenteropancreatic tumours as in vipoma and colon tumours. Indeed, it is possible to use said ligands as
30 antagonists for the inhibition of tumours growth in the VIP₁ receptor expressing tumours.

In addition, the new ligands have protecting effects in ischemia and vascular diseases and may be used as therapeutic agent in myocardial infarctions and strokes.

The new ligands according to the invention
5 may also be used in gastroenterological diseases having dysfunctions of motility, for the regeneration of the nerves, especially in post-traumatic injury, for their anti-inflammatory and anti-oxidant effects, and may therefore be used as anti-inflammatory and anti-oxidant
10 compounds.

In addition, the new ligands according to the invention may also be used for increasing the cells growth (as therapeutic agent for cicatrisation) and for the immuno-modulation of specific blood cells (as therapeutic
15 agent against auto-immune diseases and for reducing the side-effects of organs transplantations).

Another aspect of the present invention is related to a pharmaceutical composition comprising the ligands according to the invention and a (non-toxic inert)
20 therapeutically acceptable carrier material. Therefore, the new ligand according to the invention may be combined with various adequate pharmaceutical carrier to provide composition suitable for use in the treatment of bronchoconstrictive disorders such as asthma. An effective
25 dosage can be determine by one of ordinary skill in the art from the effective concentration disclosed herein. The ligand according to the invention can be used in addition to various salts such as inorganic or organic acids such as sulphuric, phosphoric, hydrochloric, hydrobromic,
30 hydroiodic, nitric, sulphamic, citric, lactic, pyruvic, oxalic, maleic, succinic, tartaric, cinnamic, acetic, trifluoroacetic, benzoic, salicylic, gluconic, ascorbic,

and related acids. The present invention and the composition according to the invention may be administered by parenteral application either intravenously, subcutaneously, intramuscularly, orally, or intranasally.

5 The present invention is also related to a diagnostic device, such as a diagnostic kit, which may comprise the ligand according to the invention, especially the labelled ligand above-described.

Said device is used to identify the above-
10 mentioned diseases or used as specific tumours marker such as neuroendocrine, gastroenteropancreatic and colon tumours, the breast and prostate cancers for metastases, the lung cancer, the ovarian cancer, the adenocarcinomas expressed in colon cancer, the squamous cell carcinomas,
15

The present invention is also related to the use of an effective amount of a selective ligand (selective agonist, antagonist or reverse agonist) of a VIP₂ receptor as an immunosuppressive and/or anti-inflammatory active
20 compound, preferably for the preparation of a medicament for the treatment and/or the prevention of a disease chosen among the group consisting of asthma, acute and chronic inflammatory diseases, digestive tract motility disorders, endocrine disorders including diabetes, low blood pression,
25 graft-vs-host disease or tissue rejection, cancer, sexual impotence or a mixture thereof.

"A selective ligand of a VIP₂ receptor" means a compound having a higher selectivity for the VIP₂ receptor than for the VIP₁ receptor, preferably the
30 mammalian VIP₂ receptor more specifically the human VIP₂ receptor.

It is meant by a "higher selectivity for the VIP₂ receptor than for the VIP₁ receptor" a ligand being 10-fold more selective for the VIP₂ receptor than for the VIP₁ receptor, preferably more than 100-fold more selective
5 for the VIP₂ receptor than for the VIP₁ receptor, more preferably more than 1000 fold more selective for the VIP₂ receptor than for the VIP₁ receptor.

Said selective ligand of the VIP₂ receptor is an agonist wherein R₁ = Ac-His, R₂ = D-Phe, R₃ = Asp,
10 R₄ = Ala or Gly, R₅ = Xaa, R₆ = Phe, R₇ = Xaa, R₈ = Glu, R₉ = Asn or Glu, R₁₀ = Tyr, R₁₁ to R₁₄ = Xaa, R₁₅ = Lys, R₁₆ = Arg or Gln, R₁₇ to R₂₂ = Xaa, R₂₃ = Leu, R₂₄ to R₂₉ = Xaa.

According to the invention, said selective
15 ligand of the VIP₂ receptor is a peptidic ligand chosen among the group consisting of the RO 25-1553 whose formula is Ac-HSDAVFTENYTKLRKQ(Nle)AAKYLNDLKKGGT-NH₂, the PG-249a whose formula is Ac-HSDAVFTENYTKLRKQ(Nle)AAKYLNDLKKGGT-NH₂ and the PG-249b whose formula is
20 Ac-HSDAVFTENYTKLRKQ(Nle)AAK(Nle)YLNNLKKGGT-NH₂.

This selective ligand of the VIP₂ receptor can be labelled as above-described for the ligand of the VIP₁ receptor and can be included in a pharmaceutical composition with an adequate pharmaceutical carrier as
25 above-described.

This compound can also be included in a diagnostic device such as a diagnostic kit for the identification of the above-mentioned diseases or used as a specific tumours marker.

The present invention will be further described in connection with the following examples and which are presented for the purpose of illustration only.

5 Brief description of the drawings

Figure 1 represents amino acids comparative sequences of VIP, [L²⁷]GRF(1-29), PG-97-268 and PG-97-269. The differences with the sequence of VIP were surrounded.

10 Figure 2 represents a summary of the binding studies of the VIP and PG-97-268 on the different receptors subtype tested.

Figure 3 represents a summary of the binding studies of the VIP and PG-97-269 on the different receptor subtypes tested.

15 Figure 4 represents amino acids comparative sequences of VIP and RO 25-1553. The differences between sequences are surrounded.

Figure 5 represents a summary of selective VIP₂ ligands on the different receptor subtypes tested.

20

Example 1

Materials and methods

Cell lines used for receptor characterisation

25 The DNA coding for the rat secretin receptor [2,15], the PACAP type I receptor [5,16] and the PACAP type II VIP₁ and VIP₂ receptors [3,17,30] were cloned into a mammalian expression vector containing the selectable neomycin phosphotransferase gene. The resulting recombinant plasmids were transfected into the CHO cell line DG44 by electroporation using a gene pulser. The selection of the

30

clones as well as their main characteristics have already been published [2,3,5,30].

Cells were maintained in α -minimal essential medium (α MEM), supplemented with 10% foetal calf serum, 5 2 mM L-glutamine, 100 μ g/ml penicillin and 100 μ g/ml streptomycin with an atmosphere of 95% air, 5% CO₂ at 37 °C. Geneticin (0.5 mg/ml) was maintained in the culture medium of the stock culture. Subcultures prepared for membrane purification were done in a medium without 10 geneticin.

LoVo cell line was obtained from ATCC (Rockville, M.D.) and cultured in Eagle's minimal essential medium (GIBCO, Gent, Belgium) supplemented with 5% foetal calf serum, 0.6 mg/ml glutamine, 200 IU/ml penicillin and 15 100 μ g/ml streptomycin.

Membrane preparation, receptor identification and adenylate cyclase activity determination

Cells were harvested with a rubber policeman 20 and pelleted by low speed centrifugation; the supernatant was discarded and the cells lysed in 1 mM NaHCO₃ and immediate freezing in liquid nitrogen. After thawing, the lysate was first centrifuged at 4 °C for 10 min at 400 x g and the supernatant was further centrifuged at 20 000 x g 25 for 10 min. The pellet, resuspended in 1 mM NaHCO₃, was used immediately on a crude membrane preparation. [¹²⁵I]VIP (specific radioactivity of 0.7 mCi/mmol), [¹²⁵I]Ac-His¹-PACAP-27 (specific radioactivity of 0.5 mCi/mmol) and [¹²⁵I]secretin (specific radioactivity of 0.3 mCi/mmol were

obtained as described previously [2,3,5]. Binding of the tracers to membranes was performed as described [2,3,5].

In all cases, the non-specific binding was defined as the residual binding in the presence of 1 μ M of the unlabelled peptide corresponding to the tracer.

Adenylate cyclase activity was determined by the Salomon et al. method [18] as previously described [6].

Peptide synthesis

All peptides were synthesised as C-terminal amides by solid phase methodology on an Automated Applied Biosystems apparatus using the Fmoc (9-fluorenylmethoxycarbonyl) strategy as previously described [6].

The peptides were cleaved and purified by reverse phase chromatography on DVB 300Å (10 x 1 cm) and by ion exchange chromatography on Mono S HR 5/5. The peptide purity was assessed (95%) by capillary electrophoresis and the sequence conformity was verified by direct sequencing and ion spray mass spectrometry.

Results

Preliminary studies on the potency and selectivity of rabbit secretin

Rabbit secretin or [L⁶, R¹⁶, L²⁷] secretin bound to the rat secretin receptor with a 6-fold lower affinity than secretin. [L⁶] secretin was indistinguishable from rabbit secretin while [R¹⁶] secretin was 2-fold less potent than secretin.

[L⁶, R¹⁶, L²⁷] secretin had a 3-fold higher affinity than secretin for the rat VIP₁ receptor. Single

substitutions in positions 6 and 16, respectively, had opposite effects : [L⁶] secretin had a 3-fold lower affinity and [Arg¹⁶] secretin a 20 fold higher affinity than secretin for the VIP₁ receptor. Thus, rabbit secretin
5 was more VIP-like than secretin and this preference was due to the substitution of Ser by Arg in position 16.

Properties of [R¹⁶] VIP and [R¹⁶] PACAP

[R¹⁶] VIP and [R¹⁶] PACAP had a 4-fold higher
10 affinity than VIP and PACAP for the VIP₁ receptor and a 10- and 4-fold higher affinity for the PACAP I receptor. [R¹⁶] secretin was also more potent than secretin on the PACAP receptor but the effect of Ser-Arg replacement could not be evaluated quantitatively, due to the low affinity of
15 secretin for that receptor.

Thus, incorporation of Arginine in position 16 of secretin, VIP and PACAP increased the peptides affinities for VIP₁ and PACAP receptors.

Furthermore, [R¹⁶] VIP and [R¹⁶] PACAP had a
20 lower affinity than VIP and PACAP for the secretin receptor.

The [R¹⁶] substituted secretin, VIP and PACAP were also tested for their capability to stimulate adenylate cyclase activity. The dose-effect curves obtained
25 on the three recombinant receptors confirmed the binding data : in cell membranes expressing the secretin receptor, [R¹⁶] secretin, [R¹⁶] VIP and [R¹⁶] PACAP were 2.5, 6.0 and 3.0 fold less potent than the corresponding unsubstituted peptides; in cell membranes expressing the VIP₁ receptor,
30 [R¹⁶] secretin, [R¹⁶] VIP and [R¹⁶] PACAP were 10, 3 and 2

fold more potent than the unsubstituted peptides; and in cell membranes expressing the PACAP receptor, [R¹⁶] secretin, [R¹⁶] VIP and [R¹⁶] PACAP were respectively 8, 4 and 2 fold more potent than the unsubstituted peptides.

5 The Inventors first observed that rabbit secretin was less potent than porcine secretin on rat secretin receptors but more potent on VIP₁ receptors. Rabbit secretin differs from porcine secretin in positions 6, 16 and 27 [14]. Substitution of the C-terminal Val²⁷-NH₂
10 by Leu²⁷-NH₂ was not investigated directly as it was considered of limited consequence : indeed, both amino acids were hydrophobic and the C-terminal 20-27 part of secretin can be replaced by the 20-27 sequence of PACAP without any modification of peptide potency. Introduction
15 of a Leu- instead of a Phe residue in position 6 of secretin decreased markedly the peptide potency on both rat secretin and VIP₁ receptors. Phe⁶, that is conserved in all members of the VIP/Sn/glucagon family of peptides, is usually considered as essential for biological activity :
20 [Tyr⁶] secretin displayed 1% of the secretin activity [19], [D-Phe⁶] secretin was inactive in in vivo models [20] and 300-fold less active than secretin in rat cardiac membranes [21], [hexahydro-Phe⁶] secretin had also a reduced activity both in vivo and in vitro [20].

25 Introduction of Arg¹⁶ instead of Ser in secretin or Gln in VIP and PACAP decreased the peptides affinities for the secretin receptor but increased their affinities for the VIP₁ and PACAP receptors. These results were rather unexpected as position 16 has never been
30 considered as important for secretin, VIP₁ or PACAP

receptor recognition. In both VIP and PACAP molecules, it is assumed that, followed a β -turn involving residues 7 to 10, the peptides adopt a continuous α -helix conformation [12,22,23] or two helical structures separated by an unidentified structure between amino acids 14 to 21 [12,24]. In secretin however, Ser¹⁶ is thought to be involved in the 13-16 reverse turn connecting the two α -helix structures 7 to 11 and 17 to 24 [24]. It is possible that the introduction of a charged amino acid in position 16 disturbs the peptides structures.

The increased affinity of the three Arg¹⁶ peptides for the VIP₁ and PACAP receptors could result either from the introduction of a new bond between the receptor and the ligand or from stabilisation of a ligand conformation that fits better in the binding pocket of the receptor.

Whatever the explanation, it appears that Arg¹⁶ interacts with the N-terminal domain of the secretin and the VIP₁ receptors : indeed, the chimeric receptors having only the N-terminal domain of secretin or VIP behave like the entire secretin and VIP₁ receptors, respectively.

Due to their potential application in human therapy, and particularly in asthma, VIP analogues with a high stability and affinity have been developed : the combination of a C-terminal extension that stabilises the terminal α -helix, of N-acetylation and of cyclisation between positions 21 to 25 led to the RO 25-1553 compound that was 10-fold more potent than VIP for tracheal smooth muscle relaxation [25]. The introduction of an Arg¹⁶ residue results in a further increase in peptide bioactivity.

Table 1

Amino acid sequence of the peptides tested.

All the peptides were carboxy terminally amidated.

		1	5	10	15	20	25																						
5	VIP	H	S	D	A	V	F	T	D	N	Y	T	R	L	R	K	Q	M	A	V	K	K	Y	L	N	S	I	L	N
	PACAP	H	S	D	G	I	F	T	D	S	Y	S	R	Y	R	K	Q	M	A	V	K	K	Y	L	A	A	V	L	
	pSn*	H	S	D	G	T	F	T	S	E	L	S	R	L	R	D	S	A	R	L	Q	R	L	L	Q	G	L	V	
	rabSn*	H	S	D	G	T	L	T	S	E	L	S	R	L	R	D	R	A	R	L	Q	R	L	L	Q	G	L	L	
	[L ⁶] Sn	H	S	G	D	T	L	T	S	E	L	S	R	L	R	D	S	A	R	L	Q	R	L	L	Q	G	L	V	
10	[R ¹⁶] Sn	H	S	D	G	T	F	T	S	E	L	S	R	L	R	D	R	A	R	L	Q	R	L	L	Q	G	L	V	

* : pSn = porcine secretin

rabSn = rabbit secretin = [L⁶, R h16, L²⁷]pSn

Example 215 Materials and methods

Peptide synthesis, cell culture and membrane preparation

The ligands VIP, PG-97-268 and PG-97-269 were synthesised as described in example 1.

Chinese hamster ovary cells (CHO cells)
 20 expressing the recombinant rat VIP₁ receptor (3), the rat VIP₂ receptor; the human VIP₂ receptor (30) and the rat PACAP type I receptor (5); the rat secretin receptor were maintained as described in the example 1.

25 Receptor identification and adenylate cyclase activation

The membrane and tracer were prepared as described in the example 1. Binding of the tracer to membrane was performed as described [2,3,5].

Adenylate cyclase activity was determined by
 30 the Salomon et al. procedure (18) as previously described (6).

Results

Characteristics of the cell lines

The characteristics of the CHO cell lines transfected with the DNA coding for the rat VIP₁-, human
5 VIP₂- and rat PACAP type I receptors were the following :
the VIP₁ r clone 3 and 16 expressed, respectively, 850 ± 50
and 100 ± 30 fmol of rat VIP₁ receptor/mg protein (3); the
VIP₂ r clone 11 expressed 210 ± 40 fmol of human VIP₂
receptor/mg protein (30) and the PACAP I r clone P2-10
10 expressed 4.6 ± 0.3 pmol of rat PACAP I receptor/mg protein
(5). mRNA was prepared from LoVo cells, reverse
transcribed into cDNA and tested by polymerase chain
reaction with appropriate primers for the presence of
VIP₁-, VIP₂- and PACAP I receptors cDNA : VIP₁ receptor
15 mRNA was only detected in LoVo cells. The Sn r clone 5
expressed 450 ± 80 fmol/mg protein (2).

Comparative effects of VIP, PG-97-268 and PG-97-269 on receptor occupation and adenylate cyclase activation

20 On CHO cell membranes expressing the rat VIP₁
recombinant receptor, VIP and the agonist PG-97-268 had an
identical IC₅₀ value of 2.0 nM but on the rat VIP₂
recombinant receptor, PG-97-268 was poorly recognised with
a IC₅₀ value of 30000 nM.

25 An identical selective profile was obtained
when we measured the inhibition of the tracer binding on
the membranes expressing the human VIP₁ and VIP₂ receptors
with IC₅₀ values of 1 and 30000 nM, respectively for the
PG-97-268.

The same selectivity was observed when measuring adenylate cyclase activity in the clone expressing the rat VIP₁ recombinant receptor and in the LoVo cells expressing the human VIP₁ receptor. The VIP/GRF hybrid stimulated adenylate cyclase activity maximally.

PG-97-268 had negligible affinities for the rat PACAP and secretin receptors.

The antagonist PG-97-269 had a good affinity for the rat VIP₁ recombinant receptor (IC_{50} : 10 nM) and the same affinity as VIP for the human VIP₁ receptor (IC_{50} : 2 nM). This VIP/GRF hybrid poorly recognised the rat VIP₂ and the human VIP₂ receptors with IC_{50} values of 2000 and 3000 nM, respectively.

PG-97-269, tested at a concentration up to 10000 nM, did not stimulate adenylate cyclase activity of any membrane preparation. PG-97-269 inhibited dose-dependently and competitively the stimulatory effect of VIP on cell membranes expressing the rat and the human VIP₁ receptors. The K_i values obtained were 15 and 2 nM for the rat and human VIP₁ receptors, respectively. PG-97-269 had negligible interactions with the rat PACAP and secretin receptors.

VIP/PACAP receptors are classified as PACAP type I receptors that had a high affinity for PACAP and a low affinity for VIP and PACAP type II receptors that had an equal high affinity for PACAP and VIP. The PACAP type II receptors were further subdivided into VIP₁ and VIP₂ receptors (34, 35). VIP₁ receptors have been cloned in rat (17) and human (26), VIP₂ receptors have been cloned in rat (28), mouse (29) and human (30). The VIP₂ receptor

corresponds to the "helodermin-preferring" receptor previously described on the basis of the relative potency of natural and synthetic analogues of VIP (31, 33).

In situ hybridisation to rat organs revealed
5 the expression of VIP₁ receptor mRNA (36) in lung large and moderate size bronchi, small intestine, thymus, liver, adrenal medulla, uterine smooth muscle and within the brain in the cerebral cortex and hippocampus. VIP₁ receptor mRNA was expressed in human epithelial cell lines (37), rat
10 pituitary cells and tumours (38) and occasionally in human brain tumours (39) and neuroblastomas (40).

The Inventors found that the new ligands PG-97-268 and PG-97-269 are highly selective agonist and antagonist of the VIP₁ receptor. Their IC₅₀ values for the
15 VIP₁ receptor are in the nanomolar range, as opposed to the range of 2000 - 3000 nM for the VIP₂ receptor.

In addition, the PG-97-268 is as efficient as VIP on the VIP₁ receptor and PG-97-269 is more efficient on the human VIP₁ receptor than on the rat VIP₁ receptor.

20 Both molecules did not recognise the PACAP I receptor and also the secretin receptor and recognised poorly the VIP₂ receptor.

PG-97-268 was a full agonist on the adenylate cyclase activity. The EC₅₀ values calculated from the
25 adenylate cyclase activity studies were in agreement with the IC₅₀ values obtained from the binding studies.

The introduction of an acetyl in position 1 and a D-Phe residue in position 2 led to an antagonist compound. PG-97-269 did not stimulate the adenylate
30 cyclase.

Diarrhoea and hypotension are the most likely side effects expected from a systemic administration of VIP or analogues (41). Diarrhoea is mediated through interaction of VIP with the enterocytes VIP₁ receptor (42).

- 5 The receptor subclass that mediates the vascular bed relaxation is not known : pharmacological studies based on the relative potency of N-terminally modified VIP analogues suggest however that these receptors are different from the liver and brain (VIP₁ and PACAP I) receptors (43). Besides
- 10 its potential therapeutic value, PG-97-268 appears to be the best tool with the RO-25-1553 (Patent EP-96870121.9) available to evaluate the contribution of each receptor subclass to a VIP mediated response.

15 Example 3

Materials and methods

Peptide synthesis and cell culture

VIP, PACAP-27 and PACAP-38 , PG96-249a and PG96-249b were synthesised as described in example 1.

- 20 Chinese hamster ovary cells (CHO cells) expressing the recombinant rat VIP₁ receptor (47), the human VIP₂ receptor (30) and the rat PACAP type I receptor (8) were maintained in as described in the example 1.

- 25 HCT 15 and LoVo cell lines (human adenocarcinoma from the colon) were obtained from ATCC (Rockville, M.D.) and cultured as in the example 1.

The SUP T1 lymphoblastic cell line was cultured in RPMI medium supplemented with 5% foetal calf serum.

The membrane preparation, receptor identification and adenylate cyclase activation were performed as described in example 1.

5 Results

Characteristics of the cell lines

The characteristics of the CHO cell lines transfected with the DNA coding for the rat VIP₁ and human VIP₂ receptors were the following : the VIP₁ r clone 3 and
10 16 expressed, respectively, 850 ± 50 and 100 ± 30 fmol of rat VIP₁ receptor/mg protein (47) and the VIP₂ r clone 11 expressed 210 ± 40 fmol of human VIP₂ receptor/mg protein (30). mRNA was prepared from SUP T1-, HCT 15 and LoVo cells, reverse transcribed into cDNA and tested by
15 polymerase chain reaction with appropriate primers for the presence of VIP₁- and VIP₂- receptors cDNA : VIP₂ receptor mRNA only was detected in SUP T1 cells and VIP₁ receptor mRNA only in HCT 15 and LoVo cells.

20 Comparative effects of VIP, PACAP-27 and RO 25-1553 on receptor occupation and adenylate cyclase activation

On CHO cell membranes expressing the rat VIP₁ recombinant receptor, the competition curves of ¹²⁵I-VIP binding inhibition were identical for the two clones
25 studied. VIP and PACAP-27 had an identical IC₅₀ value of 1.0 nM and RO 25-1553 was 100-fold less potent (IC₅₀ value of 100 nM). An identical selectivity profile was observed when measuring adenylate cyclase activity : in the clone expressing the highest receptor number and characterised by
30 low EC₅₀ value due to an amplification process linked to an

"excess" of receptors and in the clone expressing a lower receptor density, RO 25-1553 was 100-fold less potent than VIP and PACAP-27.

On CHO cell membranes expressing the human
5 recombinant VIP₂ receptor, VIP and PACAP-27 were also equipotent but 3- to 10-fold less potent than RO 25-1553 to inhibit tracer binding and to stimulate adenylate cyclase activity. On CHO cell membranes expressing a high number of rat recombinant PACAP type I receptors (P2-10 cells), VIP
10 was 300-fold less potent than PACAP-27 and 10-fold more potent than RO 25-1553.

On LoVo cell membranes that express the human VIP₁ receptor, PACAP-27 and VIP were equipotent and 600-fold more potent than RO 25-1553. RO 25-1553 had a
15 lower efficacy than VIP on adenylate cyclase activity stimulation. ¹²⁵I-VIP binding could not be valuably studied on HCT 15 cell membranes (expressing the human VIP₁ receptor), due probably to a low receptor density. However, a VIP stimulated adenylate cyclase activity was measurable.
20 RO 25-1553 was a weak stimulant. As expected for a partial agonist, the EC₅₀ value of VIP was increased in the presence of a high RO 25-1553 concentration.

On SUP T1 cell membranes, that express the human VIP₂ receptor, the results were comparable to those
25 obtained on CHO cells expressing the recombinant VIP₂ receptor : VIP and PACAP had a comparable potency lower than that of RO 25-1553.

VIP/PACAP receptors are classified as PACAP type I receptors that had a high affinity for PACAP and a
30 low affinity for VIP and PACAP type II receptors that had an equal high affinity for PACAP and VIP. The PACAP type II

receptors were further subdivided into VIP₁ and VIP₂ receptors (34, 35). VIP₁ receptors have been cloned in rat (48) and human (26), VIP₂ receptors have been cloned in rat (28), mouse (29) and human (30). The VIP₂ receptor
5 corresponds to the "helodermin-preferring" receptor previously described on the basis of the relative potency of natural and synthetic analogues of VIP (31, 33). Pharmacological studies performed on cell lines previously identified VIP₂ receptors in T lymphoblast cells (31, 49,
10 50), in a monocytic cell line (51) and in lung cancer derived cell lines (52). The mapping of the distribution of the mRNA coding for the VIP₂ receptor has been performed recently in rat tissues (53). The mRNA receptor is located in discrete brain areas, in neuroendocrine tissues, in the
15 stomach, in testis (54) but also in the terminal bronchioles. Its distribution is complementary to that of the VIP₁ receptor.

The Inventors found that the ligand RO 25-1553 is a highly selective agonist of the VIP₂
20 receptor : its IC₅₀ value for the recombinant and the SUP T1 VIP₂ receptor is approximately 0.3 nM, as opposed to 100 to 600 nM for the VIP₁ receptor and > 10 µM for the PACAP I and secretin receptors. RO 25-1553 is as efficient as VIP on the VIP₂ receptor and on the rat recombinant VIP₁
25 receptor but less efficient on the human VIP₁ receptor. Furthermore, this analogue had a lower affinity than VIP on the PACAP I receptor and also on the secretin receptor. RO 25-1553 was much more selective than the lizard peptide helodermin that previously served as reference for the
30 discrimination between VIP₁ and VIP₂ receptors. The IC₅₀

values of helodermin were indeed of 2 nM (7), 30 nM (6), 1000 nM (24) and 40 nM (25) for the VIP₂-, the VIP₁-, the PACAP I- and the secretin receptors, respectively.

The molecular basis for the high selectivity of RO 25-1553 is still speculative but several chemical modifications could participate : acetylation of the NH₂-terminus of VIP was already reported to increase peptide affinity for the VIP₂ receptor (33); a glutamic acid in position 8 (instead of aspartic acid in VIP) is also present in helodermin (57); increasing the C-terminal end of PACAP-27 derivatives led to partial agonists and antagonists with a higher affinity for the VIP₂- than for the VIP₁ receptors (58).

The present findings were unexpected considering the pharmacological profile of the ligand RO 25-1553 : in vitro, the ligand RO 25-1553 was more potent than the ligand VIP as a relaxant of isolated guinea pig tracheal - and human bronchial smooth muscle; in vivo, administered by endotracheal instillation or by aerosolisation RO 25-1553 is also more potent than VIP (25). Part of the difference between the ligands VIP and RO 25-1553 may be explained by an increased stability of the analogue. One should keep in mind however the fact that the distribution of VIP₁ and VIP₂ receptors in guinea pig and human lung has not been studied. In rat, as already mentioned, VIP₁ receptor mRNA was observed in the proximal bronchi and in the mucosa cells, and the VIP₁ receptors mRNA in the distal bronchioles.

The ligand RO 25-1553 also prevented lung inflammation during an antigen-induced pulmonary anaphylaxis; its effects on oedema, and eosinophilic

mobilisation in alveolar fluid were not reproduced by VIP (46). In that case also, the stability of the ligand RO 25-1553 could be of importance. Furthermore, VIP₁ receptors were identified in cells involved in immunity and
5 inflammation (49).

Diarrhoea and hypotension are the most likely side effects expected from a systemic administration of VIP or analogues (41). Diarrhoea is mediated through interaction of VIP with the enterocytes VIP₁ receptor (42).
10 The receptor subclass that mediates the vascular bed relaxation is not known : pharmacological studies based on the relative potency of N-terminally modified VIP analogues suggest however that these receptors are different from the liver and brain (VIP₁ and PACAP I) receptors (43). Besides
15 its potential therapeutic value, the ligand RO 25-1553 appears to be the best tool available to evaluate the contribution of each receptor subclass to a VIP mediated response.

Similar results were obtained with the ligands PG96-249a and PG96-249b (see Figure 5 enclosed).

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CLAIMS

1. Peptidic ligand having a higher selectivity for the VIP₁ receptor than for the VIP₂ receptor.
- 5 2. Ligand according to the claim 1, having a 100-fold higher selectivity for the VIP₁ receptor than for the VIP₂ receptor.
3. Ligand according to the claim 1 or 2, having a 1000-fold higher selectivity for the VIP₁ receptor
10 than for the VIP₂ receptor.
4. Ligand according to any of the preceding claims, wherein the VIP₁ receptor is a mammalian receptor, preferably a human receptor.
5. Ligand according to any of the preceding
15 claims, having in position 16 (R₁₆) a basic amino acid.
6. Ligand according to the claim 5, wherein the basic amino acid in position 16 is the Arginine (Arg).
7. Ligand according to the claim 5 or 6, having in position 4 (R₄) an amino acid chosen among the
20 group consisting of Ala, Gly, D-Ala or D-Phe.
8. Ligand according to any of the preceding claims, which is an agonist wherein R₁ = His, R₂ = Ser, R₃ = Asp, R₄ = Xaa, Ala, D-Ala or Gly, R₅ = XAA, R₆ = Phe, R₇ to R₉ = Xaa, R₁₀ = Tyr, R₁₁ to R₁₄ = Xaa, R₁₅ = Lys,
25 R₁₆ = Arg, R₁₇ to R₂₂ = Xaa, R₂₃ = Leu, R₂₄ to R₂₅ = Xaa, R₂₆ and R₂₇ = Xaa or are deleted.
9. Ligand according to any of the preceding claims, characterised in that it is an agonist having the following formula : H-S-D-A-V-F-T-N-S-Y-R-K-V-L-K-R-L-S-A-
30 R-K-L-L-Q-D-I-L-NH₂.

10. Ligand according to the claim 8 or 9, having an IC_{50} affinity value ≤ 30 nM for the VIP_1 receptor, preferably the human VIP_1 receptor.

11. Ligand according to any of the claims 1 to 7, being an antagonist wherein R_1 = Ac-His, R_2 = D-Phe, R_3 = Asp, R_4 = Xaa, Ala, D-Ala or Gly, R_5 = Xaa, R_6 = Phe, R_7 to R_9 = Xaa, R_{10} = Tyr, R_{11} to R_{14} = Xaa, R_{15} = Lys, R_{16} = Arg, R_{17} to R_{22} = Xaa, R_{23} = Leu, R_{24} to R_{25} = Xaa, R_{26} and R_{27} = Xaa or are deleted

12. Ligand according to any of the claim 11, having the following formula : Ac-H-(D-Phe)-D-A-V-F-T-N-S-Y-R-K-V-L-K-R-L-S-A-R-K-L-L-Q-D-I-L-NH₂.

13. Ligand according to the claim 11 or 12, having an IC_{50} affinity value ≤ 100 nM for the VIP_1 receptor, preferably for the human VIP_1 receptor.

14. Ligand according of any of the preceding claims, being labelled.

15. Ligand according to the claim 14, wherein the compound is labelled by a radio-active marker.

16. Ligand according to any of the claims 1 to 14, for use as a therapeutic agent.

17. Ligand according to any of the claims 1 to 14, for the treatment of a disease chosen among the group consisting of bronchoconstrictive disorders such as asthma, COPD, tumours such as neuroendocrine, gastroenteropancreatic or colon tumours, myocardial infarctions and strokes, gastroenterological diseases having dysfunctions of motility, auto-immune diseases, side-effects of organs transplantations, inflammations.

18. Ligand according to any of the claims 1 to 14, for the regeneration of the nerves, or for the increasing of the cells growth.

19. Ligand according to any of the claims 1 to 14, as anti-oxidant or anti-inflammatory compound.

20. Pharmaceutical composition comprising the ligand according to any of the claims 1 to 14 and a pharmaceutically acceptable carrier material.

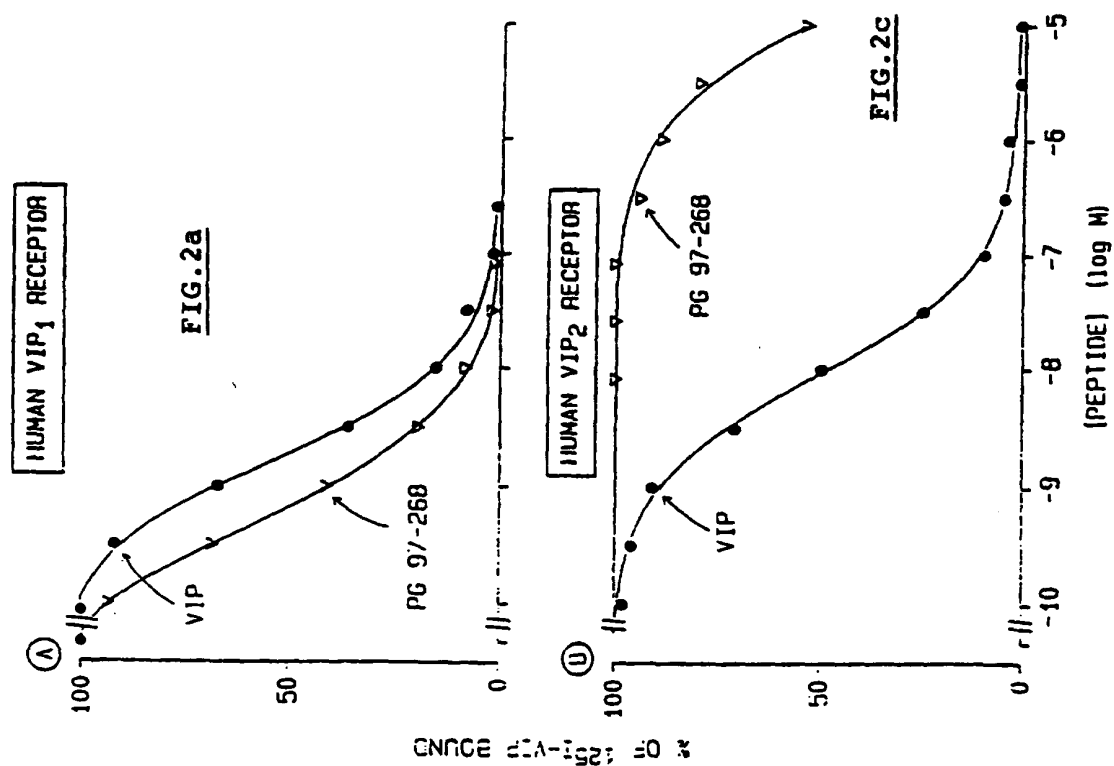
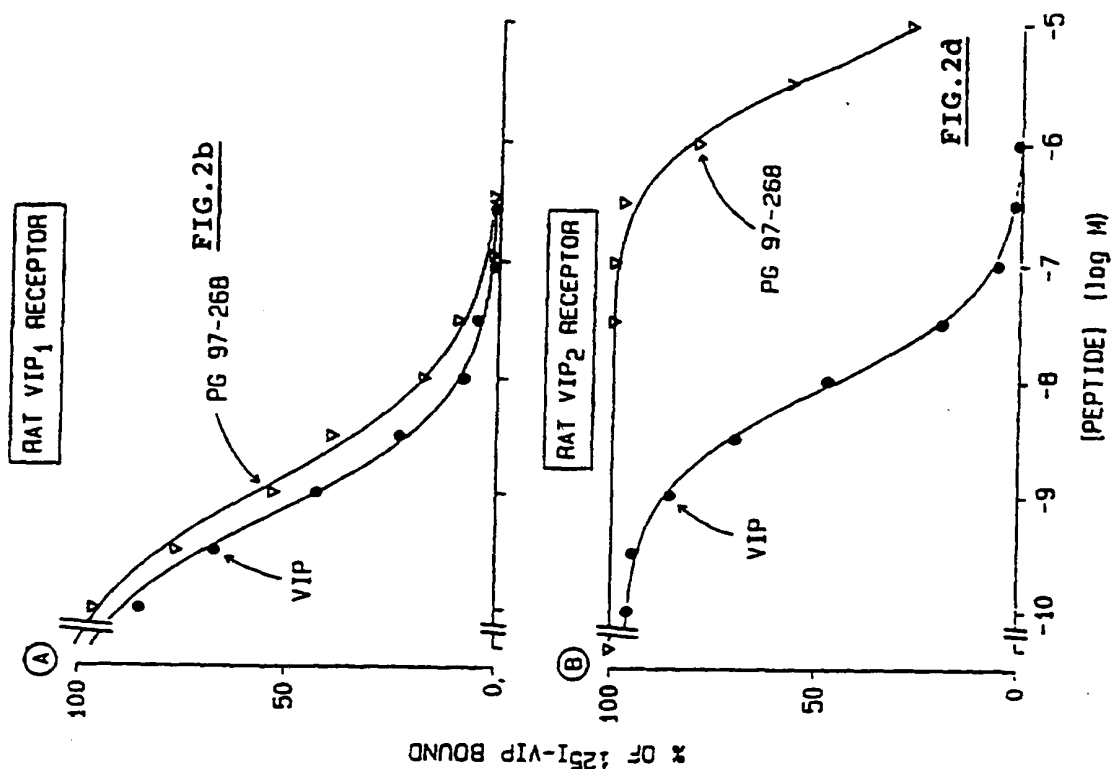
21. Diagnostic device which comprises the ligand according to the claim 14 or 15.

22. Method for recovering a compound not known to be capable of specifically binding as an antagonist or as an agonist to a VIP₁ receptor, preferably a mammal receptor, more specifically a human receptor, can specifically bind to said receptor, which comprises contacting a cell, preferably a mammalian cell or a cell extract from said cell, comprising a vector adapted for expression in said cell, which vector further comprises nucleic acid molecule which expresses said VIP₁ receptor on the cell's surface, possibly isolating a membrane fraction from the cell extract and incubating the ligand according to any of the preceding claims 1 to 13 with said cell or cell extract under conditions permitting binding of the ligand to the receptor, and with the compound, and detecting the presence of any bound compound with the VIP₁ receptor and recovering said compound.

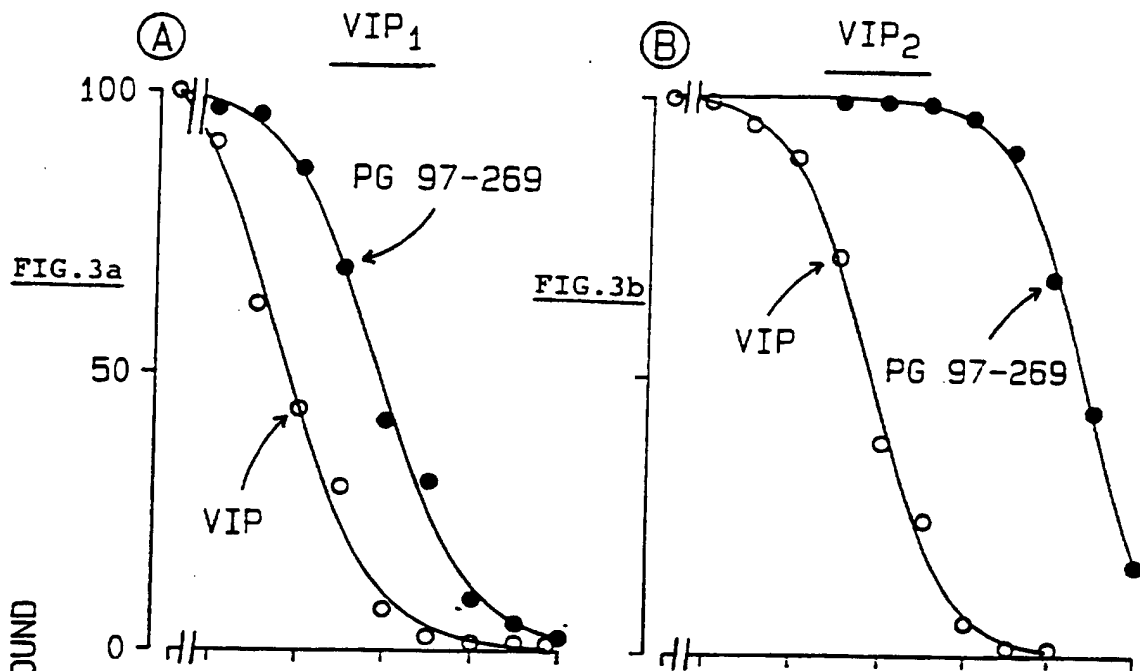
23. Compound identified by the method according to the claim 22.

24. Pharmaceutical composition comprising the compound according to the claim 23 and a pharmaceutically acceptable carrier.

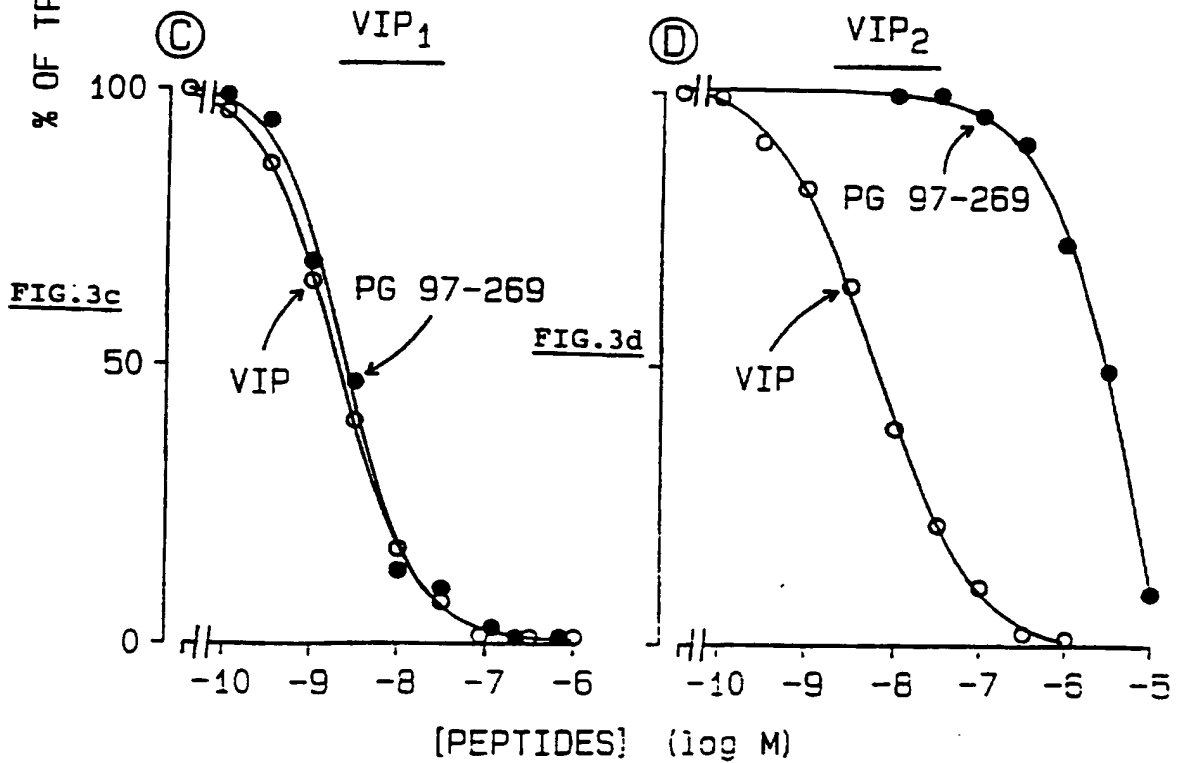
2/4

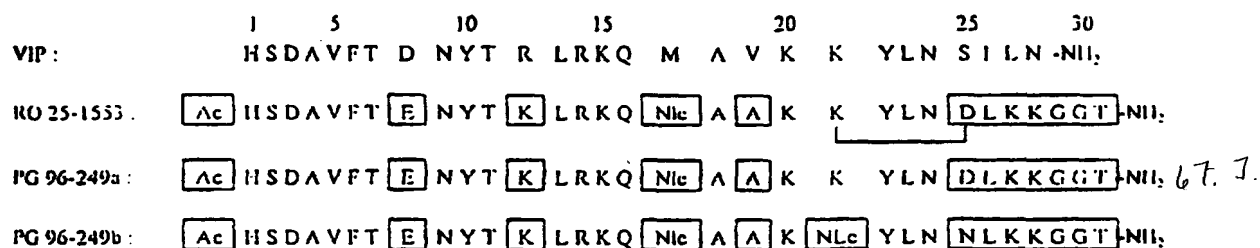


RAT RECEPTORS



HUMAN RECEPTORS





Ac = N^αacetyl; Nle = norleucine.

FIG. 4

IC₅₀ values (in nM)

	RAT VIP ₁ R	RAT VIP ₂ R	HUMAN VIP ₁ R	HUMAN VIP ₂ R
VIP	2	4	2	5
RO 25-1553	100	3	800	0.8
PG 96-249a	300	20	3000	10
PG 96-249b	300	20	3000	10

FIG. 5



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/575, A61K 38/22, G01N 33/68		A3	(11) International Publication Number: WO 98/02453
			(43) International Publication Date: 22 January 1998 (22.01.98)
(21) International Application Number: PCT/BE97/00084		(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B-1080 Brussels (BE).	
(22) International Filing Date: 15 July 1997 (15.07.97)			
(30) Priority Data: 96870092.2 15 July 1996 (15.07.96) EP		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(34) Countries for which the regional or international application was filed: AT et al.		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
96870121.9 19 September 1996 (19.09.96) EP			
(34) Countries for which the regional or international application was filed: AT et al.			
(71) Applicant (for all designated States except US): UNIVERSITE LIBRE DE BRUXELLES [BE/BE]; Avenue F.D. Roosevelt 50, B-1050 Brussels (BE).		(88) Date of publication of the international search report: 26 February 1998 (26.02.98)	
(72) Inventors; and			
(75) Inventors/Applicants (for US only): GOURLET, Philippe [BE/BE]; Avenue Guillaume Stassart 103, B-1070 Brussels (BE). ROBBERECHT, Patrick [BE/BE]; Avenue de Visée 65, B-1170 Brussels (BE). VANDERMEERS, André [BE/BE]; Avenue du Pesage 9, B-1050 Brussels (BE). WOELBROECK, Magali [BE/BE]; Rue Démosthène 63, B-1070 Brussels (BE).			
(54) Title: PEPTIDIC LIGANDS HAVING A HIGHER SELECTIVITY FOR THE VIP₁ RECEPTOR THAN FOR THE VIP₂ RECEPTOR			
<p> VIP 1 10 15 20 25 28 H S D A V F T D N Y T R L R K Q M A V K K Y L N S I L N-NH₂ [L²⁷]GRF(1-29) Y A D A I F T N S Y R K V L G Q L S A R K L L Q D I L S R-NH₂ PG 97-268 H S D A V F T N S Y R K V L K R L S A R K L L Q D I L-NH₂ PG 97-269 Ac-H X D A V F T N S Y R K V L K R L S A R K L L Q D I L-NH₂ </p> <p> Ac = N^{acetyl} X = D-Phe </p>			
(57) Abstract			
<p>The present invention is related to a peptidic ligand having a higher selectivity for the VIP₁ receptor than for the VIP₂ receptor. The present invention is also related to the diagnostic device, the pharmaceutical composition comprising said ligand and to their use.</p>			

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/575 A61K38/22 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>P. ROBBERECHT AND P. GOURLET: "Intérêt des récepteurs recombinants dans l'élaboration d'agonistes et d'antagonistes des récepteurs du VIP et du PACAP"</p> <p>JOURNAL DE PHARMACIE DE BELGIQUE, vol. 51, no. 3, May 1996 - June 1996, pages 165-169, XP002049504 see table II</p> <p style="text-align: center;">--- -/--</p>	1-4, 16-24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

8 December 1997

Date of mailing of the international search report

09.01.98

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Fuhr, C

INTERNATIONAL SEARCH REPORT

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PCT/BE 97/00084

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	P. GOURLET ET AL.: "The Long-Acting Vasoactive Intestinal Polypeptide Agonist R0 25-1553 is Highly Selective of the VIP2 Receptor Subclass" PEPTIDES, vol. 18, no. 3, 1997, pages 403-408, XP002049505 see page 406, right-hand column, paragraph 3 - page 407, right-hand column, last paragraph; figure 1 ---	1-4
A	M. SVOBODA ET AL.: "Molecular Cloning and Functional Characterization of a Human VIP Receptor from SUP-T1 Lymphoblasts" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 205, no. 3, 30 December 1994, ORLANDO, FL US, pages 1617-1624, XP002049506 cited in the application see page 1621, paragraph 1 - page 1623, paragraph 1 ---	1-4
A	EP 0 536 741 A (HOFFMANN LA ROCHE) 14 April 1993 cited in the application see page 23, line 35 - line 37; claims; examples -----	1-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BE 97/00084

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 23-24
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
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3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interns 31 Application No

PCT/BE 97/00084

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